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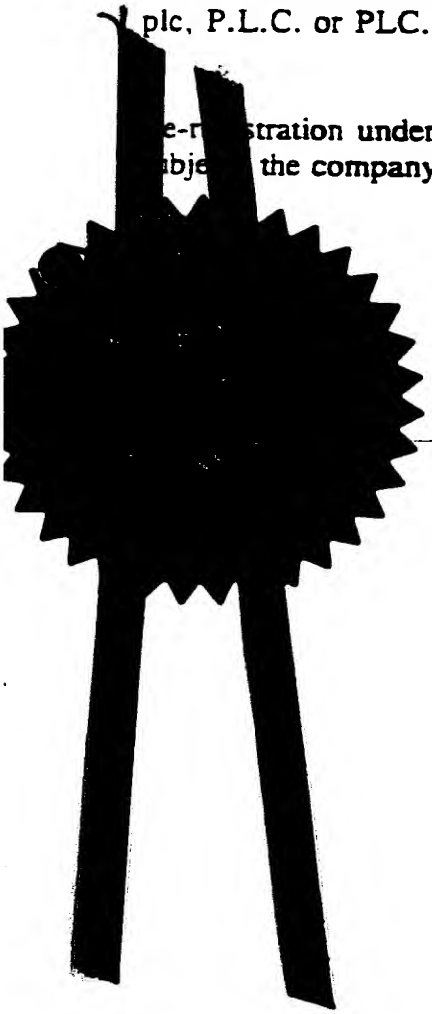

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Signed A M Brewer.

Dated 13/11/1998.

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11NOV97 E316266-1 002732
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# Request for grant of a patent

*(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)*

1. Your reference — A950 — 07 NOV 1997

2. Patent application number *(The Patent Office will fill in this part)* — 9723669.9

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

ABERDEEN UNIVERSITY
AURIS BUSINESS CENTRE
23 ST MACHAR DRIVE
ABERDEEN, AB12 1RY
UNITED KINGDOM

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — UNITED KINGDOM — 29395100 4

4. Title of the invention — SKIN PENETRATION ENHANCING COMPONENTS

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

ABLETT & STEBBING
45 LANCASTER MEWS
LANCASTER GATE
LONDON
W2 3QQ

Patents ADP number *(if you know it)* — 6551001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

| Country | Priority application number *(if you know it)* | Date of filing *(day / month / year)* |
|---|---|---|
| | | |

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

| Number of earlier application | Date of filing *(day / month / year)* |
|---|---|
| | |

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

*a) any applicant named in part 3 is not an inventor, or*
*b) there is an inventor who is not named as an applicant, or*
*c) any named applicant is a corporate body.*
*See note (d))*

Yes

9. Enter the number of sheet any of the following items you are filing with this form. Do not count copies of the same document

| | |
|---|---|
| Continuation sheets of this form | 0 |
| Description | 6 |
| Claim(s) | 2 |
| Abstract | 0 |
| Drawing(s) | 4 + 4 |

10. If you are also filing any of the following, state how many against each item.

| | |
|---|---|
| Priority documents | 0 |
| Translations of priority documents | 0 |
| Statement of inventorship and right to grant of a patent *(Patents Form 7/77)* | 0 |
| Request for preliminary examination and search *(Patents Form 9/77)* | 0 |
| Request for substantive examination *(Patents Form 10/77)* | 0 |
| Any other documents *(please specify)* | NONE |

11. I/We request the grant of a patent on the basis of this application.

Signature ABLETT & STEBBING Date 7 November 1997

12. Name and daytime telephone number of person to contact in the United Kingdom

GK ABLETT/PJH STEBBING (0171-262-4108)

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# SKIN PENETRATION ENHANCING COMPONENTS

This present invention relates to an effective treatment for psoriasis and other dermatological conditions using a topically applied immunosuppressive agent; preferably one which does not appear in the blood at any significant level.

Dermatological conditions can be uncomfortable and embarrassing for the patient, so an effective safe treatment is required. Some dermatological conditions are caused by an overactive immune system, examples are psoriasis, alopecia, lichen planus, lupus erythematosus, pyoderma gangrenosum, vitiligo and graft versus host disease. Others can be due to bacterial or pustular skin infections.

Dermatological conditions caused by an overactive immune system can be treated by immunosuppressive macrolides, for example sirolimus, FK-506 or SDZ ASM 981. Those that are caused by bacteria or are deeper skin infections, such as acne vulgaris and hidranitis suppcurativa, can be treated by macrolide antibiotics, for example erythromycin, azithromycin and clarithromycin. The above agents may be applied orally by means of topical creams and lotions or taken orally.

Psoriasis affects 2.4% of the population and the current understanding of the pathogenesis of the disease is that it is driven initially by immunocytes. These and keratinocytes are mutually stimulated and activated through the production of cytokines, TGFa, IL-6 and IL-8 from lymphocytes. This leads to a hyperproliferative epidermis with rapid 36 hour cycling of the transient amplifying compartment of keratinocytes.

FK506 is a macrolide antibiotic which shows part homology with sirolimus. Research in models has shown that it has some efficacy in the topical therapy of contact dermatitis, atopic eczema and to a lesser degree psoriasis. Cyclosporin is also

known to be effective in treating a wide range of skin diseases. However the usefulness of these drugs is limited by their potential side effects resulting from systemic administration.

Other forms of treatment of dermatological conditions may include using topical steroids but these have undesirable effects such as irreversible atrophy and purpura.

It is known that immunosuppressive agents taken orally and steroids applied topically can be used to treat dermatological conditions, such as psoriasis. However, they are often non-specific in their action which leads to undesirable side effects. Thus it would be desirable to develop a topical immunosuppressive agent for application which preferentially treats the diseased sites and avoids significant systemic exposure; so reducing harmful side effects.

Sirolimus is a macrolide antibiotic produced by the organism *Streptomyces hygroscopicus*, it is known to have potent immunosuppressive activities. Sirolimus acts through specific binding of a family of cytosolic immunophilins called the FK binding proteins (FKBP). The sirolimus FKBP complex acts in two stages. Firstly by blocking the phosphorylation activation of p70 s6 kinase, an enzyme acting on the 40S ribosomal subunit s6 protein, thereby reducing the translation of ribosomal proteins and elongation factors required for protein synthesis. Secondly it inhibits enzyme activity of the cyclin dependent kinase cdK-cyclin E complex which forms one of the tight controls of the G1/S transition in cell division by inhibiting the normal decline of the p27 cdk inhibitor which would follow IL-2 stimulation. Sirolimus has an advantage over other immunosuppressive agents in the treatment of psoriasis as it has an inhibitory effect on keratinocyte proliferation. *In vitro* experiments have shown that this inhibitory effect takes place at concentrations ranging from 3-10$\mu$g/ml. A broader range may be employed for

example 1 to 20μg/ml, but the more efficacious range is 5-8μg/ml.

According to the first aspect of the invention, there is provided a topical immunosuppressive agent for the treatment of a dermatological condition comprising a macrolide antibiotic or immunosuppressive macrolide characterised by a permeation enhancer; the permeation enhancer and the macrolide antibiotic or immunosuppressive macrolide being present in related amounts, such that when applied to the skin a minimal systemic effect is produced on application.

Preferably the macrolide antibiotic is selected from erythromycin, azithromycin or clarithromycin. These macrolide antibiotics are effective for treating pustular and bacterial skin infections such as acne vulgaris.

Conveinently the immunosuppressive macrolide is selected from sirolimus, FK-506 or SDZ ASM 981. Sirolimus is a favoured alternative because it is also an effective immunosuppressive macrolide which is useful in the microbiological preservation of the formulation. The microbiological properties of sirolmus are also helpful in the treatment of scalp and flexural psoriasis, seborrhoeic dermatitis and in secondarily infected lesions of atopic eczema.

In preferred embodiments the permeation enhancer may be an alkanoic or alkenic acid such as capric acid, octanoic acid, oleic acid or acids of intermediate chain length. The permeation enhancer is required to aid the penetration of the immunosuppressive macrolide or macrolide antibiotic through the stratum corneum, the principal barrier to the penetration of drugs. The stratum corneum is an aggregate of the stacked, flattened skeletons of keratin filled cells interspersed with lipid monolayer structures and water. The addition of the permeation enhancer to the formulation results in the partial disruption of the barrier components, particularly the lipid

structures. A gradient of the drug can then be produced across the stratum corneum particularly, which facilitates the diffusion of the immunosuppressive macrolide or macrolide antibiotic across the stratum corneum into the living epidermis. The relative concentrations of the antibiotic and the permeation enhancer are chosen so that only partial penetration of the skin occurs; the macrolide antibiotics or immunosuppressive macrolides reach the required areas but significant absorption of the drugs into the systemic circulation is avoided thus reducing the likelihood of any side effects.

Conveniently the permeation enhancer is used in conjunction with a solvent system which uses an aromatic alcohol, or a benzene derivative, with or without a mixture of monoglycerides and a fatty acid ester (e.g. isopropyl myristate). Other solvents used, include benzaldehyde, benzyl benzoate and acetone. The combination of solvent and permeation enhancer optimises the passage of the immunosuppressive macrolide and macrolide antibiotic across the stratum corneum.

Preferably a thickening agent is present in the formulation. If the formulation is to be used topically, it should be in an appropriate consistency. Therefore, thickening agents such as cetostearyl alcohol or white soft paraffin may be added. These can reduce the penetration of the immunosuppressive agent but they are required for effective application.

The invention will now be described, by way of illustration only, with reference to the following examples, tables and figures accompanying the specification

Figure 1 is a graphical representation of the effect on the flux of sirolimus through the stratum corneum by varying the capric acid and benzyl alcohol ratio.

Figure 2 is a graphical representation of the effect on the flux of sirolimus through the stratum corneum by varying the octanoic acid and benzyl alcohol ratio.

Figure 3 is a graphical representation of the effect on the flux of sirolimus through the stratum corneum by varying the oleic acid and benzyl alcohol ratio.

Figure 4 is a graphical representation of the effect on the flux of sirolimus through the stratum corneum by varying the sirolimus concentration while keeping the capric acid to benzyl acid ratio constant.

Figures 1 to 4 were obtained by *in vitro* experimentation. The results were used to optimize the sirolimus concentration and the ratio of permeation enhancer and solvent used in *in vivo* experiments.

Example 1

A formulation comprising a vehicle of capric acid (50%) with benzyl alcohol (50%) was added to sirolimus (8%). This was tested in single application experiments on three individuals with normal skin. Venous blood samples were taken at 4, 7 and 24 hours after application and no significant levels of sirolimus were detected using MSGCMS. This latter assay is able to detect sirolimus levels down to 0.1ng/ml.
In parallel, skin biopsies were taken from 2 subjects after 7 hours, the biopsy samples were glued to a glass slide and serially sectioned in 2 layers each and extracted with acetonitrile. The results are given in Table 1.

Table 1 shows the tissue concentrations of sirolimus 7 hours after application of capric acid:benzyl alcohol (50:50) containing sirolimus at 8%.

| Level of skin 1=surface | Sirolimus concentration µg/mg | | | |
|---|---|---|---|---|
| | A | B | C | D |
| 1 | 0.059 | 0.288 | 0.301 | 0.216 |
| 2 | Not done | 0.108 | 0.144 | 0.126 |
| 3 | 0.255 | 0.173 | 0.339 | 0.256 |
| 4 | 0.239 | 0.214 | 0.370 | 0.241 |

Example 2

A formulation comprising isopropyl myristate 40%, benzyl alcohol 10%, capric acid 50% and sirolimus (2.2%; was tested in single application experiments on three individuals with normal skin. Venous blood samples were taken at 4, 7 and 24 hours after application and no significant levels of sirolimus were detected using MSGCMS.

After 7 hours biopsy samples were taken from two of the individuals. These were bisected in parallel with the surface to give an upper and lower half, roughly corresponding to the epidermis and dermis. The skin was homogenised with acetonitrile and sirolimus concentration was determined by HPLC. The results are given in Table 2

Table 2 shows the tissue concentrations of sirolimus 7 hours after application of capric acid:isopropyl myristate:benzyl alcohol (50:40:10) containing sirolimus at 2.2%.

| Level of skin segment | Sirolimus Concentration µg/mg | |
|---|---|---|
| | Subject A | Subject B |
| Upper (1) | 0 | 1.5 |
| Lower (2) | 0.333 | 0.5 |

CLAIMS

1) A topical immunosuppressive agent for the treatment of a dermatological condition comprising a macrolide antibiotic or an immunosuppressive macrolide characterised by a permeation enhancer; the permeation enhancer and the macrolide antibiotic being present in relative amounts such that when applied to the skin a minimal systemic effect is produced.

2) An agent according to claim 1 wherein the macrolide antibiotic is selected from erythromycin, azithromycin or clarithromycin.

3) An agent according to claim 1 wherein the immunosuppressive macrolide is selected from sirolimus, FK506 or SDZ ASM 981.

4) An agent according to any preceding claim wherein the permeation enhancer is an alkanoic acid or alkenic acid.

5) An agent according to claim 4 wherein the alkanoic acid or alkenic acid is selected from capric acid, octanoic acid, oleic acid or acids of intermediate chain length.

6) An agent according to any preceding claim wherein the dermatological condition is selected from psoriasis, alopecia, eczema dermatitis, lichen planus, lupus erthematosus, pyoderma gangrenosum, vitiligo, graft versus host disease, pustular skin infections, bacterial skin infections or acne vulgaris.

7) An agent according to any preceding claim wherein the concentration of macrolide antibiotic or immunosuppressive macrolide is 0.01%-10% by weight.

8) An agent according to any preceding claim wherein the concentration of the permeation enhancer is 0.1%-60% by weight.

9) An agent according to any preceding claim wherein the permeation enhancer is used in conjunction with a solvent system.

10) An agent according to claim 9 wherein the solvent system is an aromatic alcohol or a benzene derivative, with or without a mixture of monoglycerides and a fatty acid ester.

11) An agent according to any preceding claim wherein the concentration of the solvent system is 5% to 90%.

12) An agent according to any preceding claim further comprising a thickening agent.

13) An agent according to claim 12 wherein the thickening agent is selected from white soft paraffin, cetostearyl alcohol, yellow soft paraffin, cetyl alcohol, steryl alcohol, divalent carboxylic acid soaps and carnauber wax.

14) The use in the manufacture of a topical composition for for the treatment of a dermatological condition of a macrolide antibiotic or an immunosuppressive macrolide characterised by a permeation enhancer; the permeation enhancer and the macrolide antibiotic or the immunosuppressive macrolide being present in relative amounts such that when applied to the skin a minimal systemic effect is produced.

15) The use of claim 14 wherein the macrolide antibiotic or immunosuppressive macrolide is present at 0.01% to 10% by weight of the composition.

Figure 1

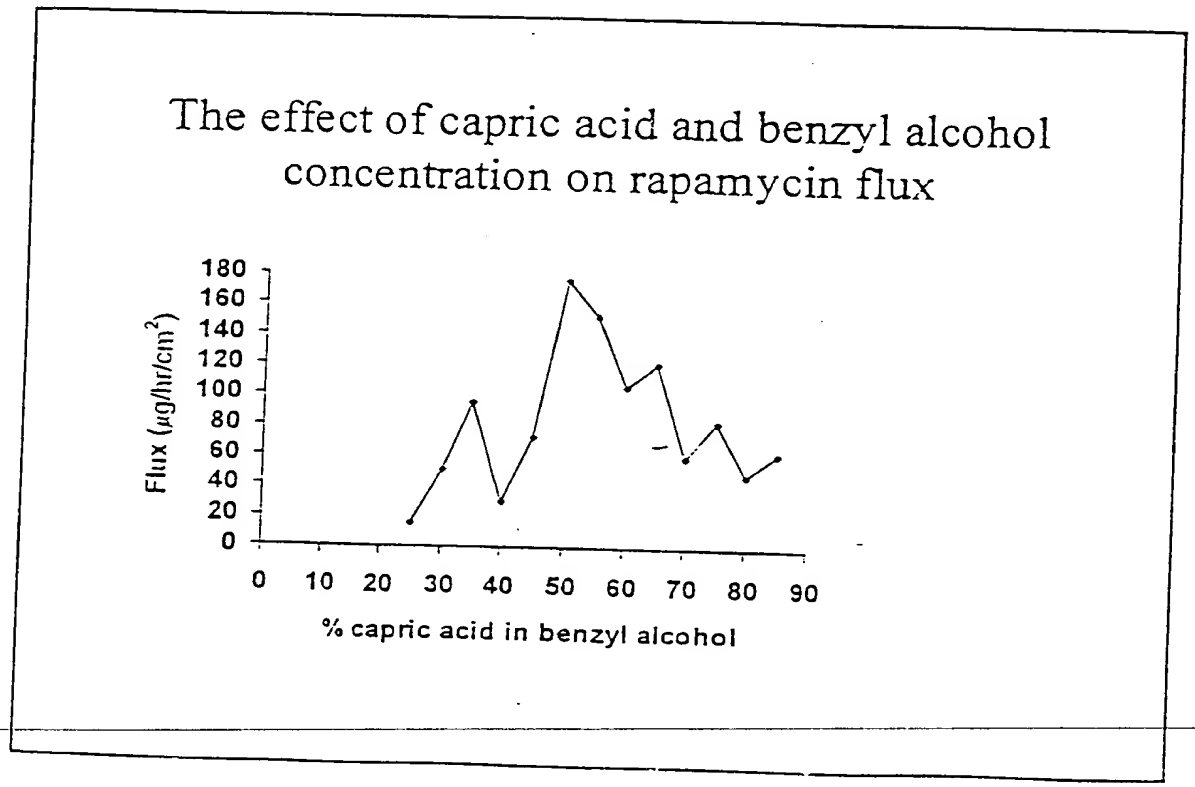

The effect of capric acid and benzyl alcohol concentration on rapamycin flux
Flux (µg/hr/cm²)
180
160
140
120
100
80
60
40
20
0
0 10 20 30 40 50 60 70 80 90
% capric acid in benzyl alcohol

Figure 2

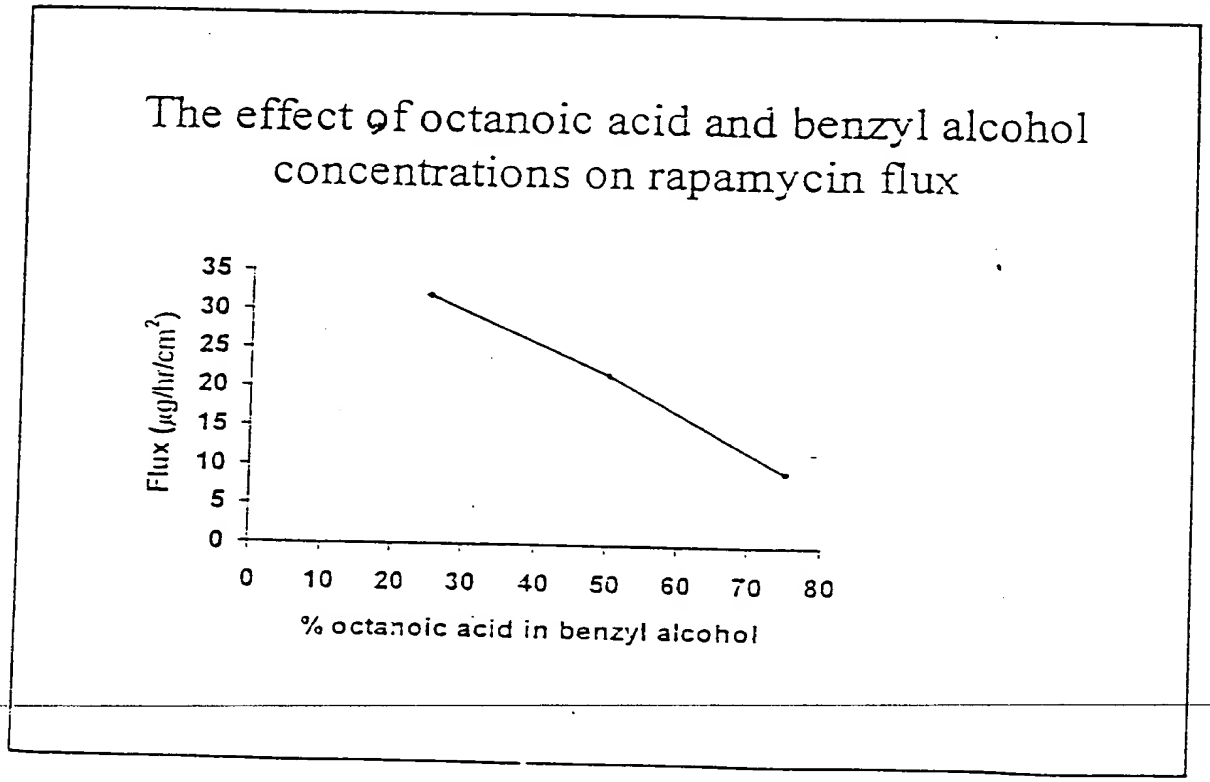

The effect of octanoic acid and benzyl alcohol concentrations on rapamycin flux
Flux (μg/hr/cm²)
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
% octanoic acid in benzyl alcohol

Figure 3

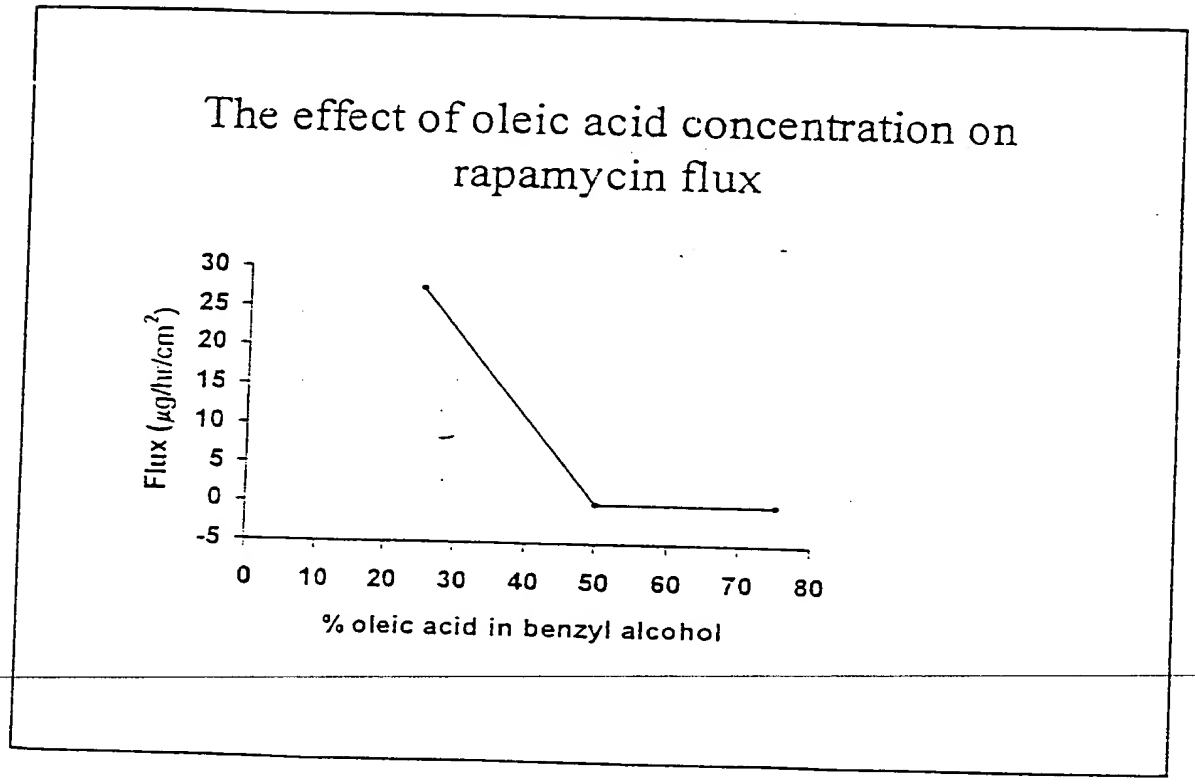
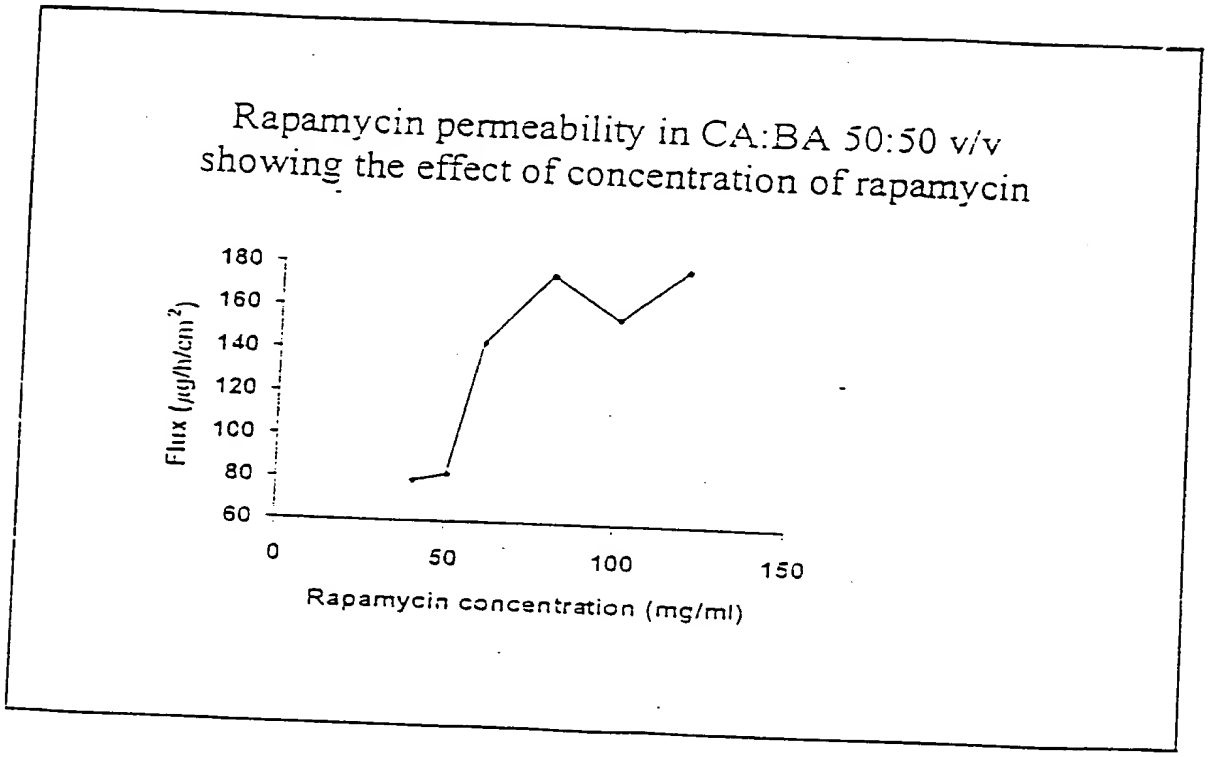

The effect of oleic acid concentration on rapamycin flux
Flux (µg/hr/cm²)
30
25
20
15
10
5
0
-5
0
10
20
30
40
50
60
70
80
% oleic acid in benzyl alcohol

Figure 4


Rapamycin permeability in CA:BA 50:50 v/v
showing the effect of concentration of rapamycin
Flux (µg/h/cm²)
180
160
140
120
100
80
60
0
50
100
150
Rapamycin concentration (mg/ml)